

## CLAIMS

What is claimed is:

1. A method of introducing heterologous DNA into a non-plant host cell thereby producing a gene product in said cell, said method comprising:
  - a) inserting heterologous DNA encoding said gene product into a unique restriction endonuclease cleavage site of a vector, said vector comprising:
    - i) a backbone which includes a first origin of replication capable of maintaining heterologous DNA as a single copy in *Escherichia coli* host cell, and which further includes a second origin of replication capable of maintaining heterologous DNA as a single copy in an *Agrobacterium tumefaciens* host cell;
    - ii) a unique restriction endonuclease cleavage site for insertion of heterologous DNA; and
    - iii) left and right *Agrobacterium* T-DNA border sequences flanking said unique restriction endonuclease cleavage site, said left and right T-DNA border sequences allowing introduction of heterologous DNA located between left and right T-DNA border sequences into a non-plant cell;
  - b) transforming a non-plant cell so as to introduce said heterologous DNA into said cell; and

c) expressing said heterologous DNA in said non-plant cell so as to produce the gene product encoded by said heterologous DNA into said cell.

2. The method of claim 1, wherein said host cell is a yeast cell or a filamentous fungus.

3. The method of claim 2, wherein the yeast cell is *Saccharomyces cerevisiae* or *Kluyveromyces lactis*.

4. The method of claim 2 wherein the filamentous fungus is from the genus *Aspergillus*.

5. A method of producing a gene product in a non-plant host cell, said method comprising:

a) inserting heterologous DNA encoding said gene product into a unique restriction endonuclease cleavage site of a vector, said vector comprising:

i) a backbone which includes a first origin of replication capable of maintaining heterologous DNA as a single copy in *Escherichia coli* host cell;

ii) a unique restriction endonuclease cleavage site for insertion of heterologous DNA; and

iii) left and right *Agrobacterium* T-DNA border sequences flanking said unique restriction endonuclease cleavage site, said left and right T-DNA border sequences allowing introduction of heterologous DNA located between left and right T-DNA border sequences into a non-plant host cell;

b) introducing the resulting vector, into said non-plant host cell; and

c) expressing said heterologous DNA in said non-plant host cell so as to produce the gene product encoded by said heterologous DNA.

6. The method of claim 5, wherein said vector further includes a second origin of replication capable of maintaining heterologous DNA as a single copy in an *Agrobacterium tumefaciens* host cell.
7. The method of claim 5, wherein the non-plant host cell is *Escherichia coli*.
8. The method of claim 5, wherein the non-plant host cell is a non-plant eukaryotic cell.
9. The method of claim 8, wherein the non-plant eukaryotic cell is a yeast cell.
10. The method of claim 8, wherein the non-plant eukaryotic cell is a mammalian cell.
11. The method of claim 1 or 5, wherein the heterologous DNA is obtained from genomic DNA of prokaryotic cells.
12. The method of claim 1 or 5, wherein the heterologous DNA is obtained from genomic DNA of eukaryotic cells.
13. The method of claim 1 or 5, wherein said first origin of replication comprises an F origin from *Escherichia coli*.
14. The method of claim 1 or 5, wherein said second origin of replication comprises an Ri origin from *Agrobacterium rhizogenes*.
15. The method of claim 6, wherein first origin of replication comprises an F origin from *Escherichia coli* and said second origin of replication comprises an Ri origin from *Agrobacterium rhizogenes*.
16. The method of claim 1 or 5, wherein said unique restriction endonuclease cleavage site comprises a BamHI cleavage site.

17. The method of claim 1 or 5, wherein said left and right T-DNA border sequences are derived from TL-DNA of octopine plasmid pTiA6.
18. The method of claim 1 or 5, further comprising a selection marker for incorporation of heterologous DNA into said vector.
19. The method of claim 1 or 5, wherein said selection marker comprises a sacB gene, and wherein when heterologous DNA is inserted into unique restriction endonuclease cleavage site of said vector, said sacB gene is inactivated.
20. The method of claim 1 or 5, further comprising a selection marker for introduction of said heterologous DNA into *Escherichia coli*.
21. The method of claim 1 or 5, wherein said selection marker comprises a kanamycin resistance gene.
22. The method of claim 6, further comprising a selection marker for the introduction of said heterologous DNA into *Agrobacterium tumefaciens*.
23. The method of claim 1 or 5, further comprising a selection marker for introduction of said heterologous DNA into a non-plant eukaryotic cell, said selection marker located between said left and right T-DNA border sequences.
24. The method of claim 1 or 5, wherein said selection marker is located adjacent to said left T-DNA border sequence.
25. The method of claim 21, wherein said kanamycin resistance gene comprises a GUS-NPTII gene.
26. The method of claim 20, wherein said selection marker comprises a hygromycin resistance gene.

Rule 126  
MMA 3/15/03

27-25. The method of claim 1 or 5, wherein said backbone further comprises an origin of conjugal transfer.

28-26. <sup>27</sup>The method of claim ~~25~~, wherein said origin of conjugal transfer comprises an oriT origin from plasmid RK2.

29-27. A non-plant eukaryotic host cell containing a vector, said vector comprising:

- a) a backbone which includes a first origin of replication capable of maintaining heterologous DNA as a single copy in *Escherichia coli* host cell;
- b) a unique restriction endonuclease cleavage site for insertion of heterologous DNA; and
- c) left and right *Agrobacterium* T-DNA border sequences flanking said unique restriction endonuclease cleavage site, said left and right T-DNA border sequences allowing introduction of heterologous DNA located between left and right T-DNA border sequences into a non-plant host cell;
- d) a heterologous DNA inserted at said unique restriction endonuclease cleavage site; and
- e) a second origin of replication capable of maintaining heterologous DNA as a single copy in an *Agrobacterium tumefaciens* host cell.

30-28. The non-plant eukaryotic host cell of claim <sup>29</sup>~~27~~, wherein the host cell is a yeast cell.

31-29. The non-plant eukaryotic host cell of claim <sup>29</sup>~~27~~, wherein the host cell is a mammalian cell.

32-30. The host cell of claim <sup>29</sup>~~27~~, wherein the heterologous DNA is from a eukaryotic cell.

33-31. The host cell of claim <sup>29</sup>~~27~~, wherein the heterologous DNA is from a prokaryotic cell.

34/32.

A method of isolating a DNA encoding a desired gene product from a genomic library of DNA comprising:

- a) inserting heterologous DNA from a genomic library of DNA into a vector, said vector comprising:
  - i) a backbone which includes a first origin of replication capable of maintaining heterologous DNA as a single copy in *Escherichia coli* host cell;
  - ii) a unique restriction endonuclease cleavage site for insertion of heterologous DNA; and
  - iii) left and right *Agrobacterium* T-DNA border sequences flanking said unique restriction endonuclease cleavage site, said left and right T-DNA border sequences allowing introduction of heterologous DNA located between left and right T-DNA border sequences into a non-plant host cell;
- b) introducing the resulting vector, into said non-plant host cell; and
- c) expressing said heterologous DNA in said non-plant host cell so as to produce the gene product encoded by said heterologous DNA,
- d) screening the cultured host cells for those cells that express the desired gene product, and
- e) isolating the DNA encoding the desired gene product from those cells that express the desired gene product.

35 ~~33~~

<sup>34</sup>  
The method of claim ~~32~~, wherein said vector further includes a second origin of replication capable of maintaining heterologous DNA as a single copy in an *Agrobacterium tumefaciens* host cell.

36 ~~34~~

<sup>34</sup>  
The method of claim ~~32~~, wherein the host cell is *Escherichia coli*.

37 ~~35~~

<sup>34</sup>  
The method of claim ~~32~~, wherein the host cell is a non-plant eukaryotic cell.

38 ~~36~~

<sup>34</sup>  
The method of claim ~~32~~, wherein the non-plant eukaryotic host cell is a yeast cell.

39 ~~37~~

<sup>34</sup>  
The method of claim ~~32~~, wherein the non-plant eukaryotic host cell is a mammalian cell.

40 ~~38~~

<sup>34</sup>  
The host cell of claim ~~32~~, wherein the genomic library is obtained from prokaryotic cells.

41 ~~39~~

<sup>34</sup>  
The host cell of claim ~~32~~, wherein the genomic library is obtained from eukaryotic cells.